Abstract

A study of enamel remineralization by

bioactive glass/chitosan/zinc oxide composite nanoparticles

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【Introduction】

The bonding of the multi-bracket appliance to the enamel surface requires the decalcification of the tooth by etching. In addition, plaque accumulation around the bracket and the deterioration of the oral hygiene environment increase the risk of dental caries. Therefore, the development of functional materials that suppress enamel demineralization and promote remineralization is desired.

Particularly. development research on bioactive ceramics in the medical field is active. Bioactive glass slowly releases various ions present in the body, thereby promoting remineralization and acid buffering. Because of that, the applications such as dental implants and toothpaste have been investigated. Chitosan extracted from crustacean chitin is expected to be applied to dental biomaterials because it has excellent biodegradability, biocompatibility and caries-preventing effect.

In recent years, with the progress of nanotechnology, these functional materials have been studied for enhancing size-dependent effects and imparting functionality by nanoparticulation. Among them, the antibacterial properties of ceramic nanoparticles such as zinc oxide are remarkably useful. Today, there is concerned about the outbreak of anti-drug-resistant bacteria due to lifestyle changes and high doses of drugs in the medical field. It is attracting attention as a new antibacterial material.

The aim of this study was to investigate the remineralization effect of enamel and the antibacterial effect against cariogenic bacteria in bioactive glass, chitosan, and zinc oxide composite nanoparticles.

【Material and Methods】

1. Material preparation

1)Bioactive glass micro/nano particles

The bioactive glass microparticles(μB) with composition of SiO_2, Na_2O, CaO, and P_2O_5 was made by melting method at the 1550℃. The BG was ground in a ball mill to obtain a particle size is 10 μm or less.

The bioactive glass nanoparticles(nB) were made by sol-gel method. Tetraethyl orthosilicate, $NaNO_3$, $Ca(NO_3)_2$ were dispersed in a mixture of deionized water and ethanol, and the pH was adjusted to 1 to 2. The solution was stirred until it became transparent, then dropped into ammoniated deionized water containing ammonium diammonium phosphate. During the dripping process, the pH was kept at 10 to 11. After centrifugation, the supernatant was discarded, the precipitate was lyophilized, and calcinated at 700 ° C.

2)Chitosan nanoparticles

Chitosan was dissolved at 0.5% (w/v) with 1% (v/v) HOAc, then adjusted to pH4.6–4.8 with NaOH. Chitosan nanoparticles(C) formed spontaneously upon addition of an aqueous tripolyphosphate solution (0.25%, w/v) to chitosan solution under magnetic stirring. Nanoparticles were purified by centrifugation.

3)zinc oxide nanoparticles

Zinc oxide nanoparticles(Z) purchased from Sigma-Aldrich (721077). It was dispersed in pure water and the average particle size was $\leq$40 nm.

2. Material characterization

The produced nanoparticles were confirmed for morphology and structure using Scanning electron microscope (JSM-7800F, JEOL) (SEM) and Transmission Electron Microscope (JEM-2100F, JEOL) (TEM). To examined in more detail, the specific surface area was identified by the gas adsorption measurement (Autosorb 6AG, Yuasa Ionics), and the particle dispersion stability were evaluated by the zeta potential measurement (Delsa nano HC, Beckman Coulter). Furthermore, crystal structure analysis by X-ray diffraction (Rint-2500, Rigaku) (XRD) and acid buffering capacity test were performed for nBG.

3. Evaluation of remineralization ability of enamel

1) Preparation of embedded specimen

After enamel was cut out from human extracted teeth, it was embedded in epoxy resin (Epofix, Struers). The sample after embedding was polished by a polishing machine (ML-110N, Maruto).

2) Immersion test

Fluoride-containing gel (Check-Up gel, Lion) (F), nB, nBC, nBZ,nBCZ and μB were used as materials to be applied to enamel samples. The enamel samples were decalcified by 35% phosphoric acid etching, a remineralization test was carried out for 28 days, in which immersion in a remineralization solution (pH 6.8) for 7 hours and immersion in the each material suspension for 1 hour were performed alternately. As a control, samples only immersed in the remineralization solution were also prepared (Con).

3) Nanoindentation testing

Nanoindentation testing of the enamel surfaces (ENT-1100a, ELIONIX) was carried out at 28°C using 10 and 100 mN loads before and after demineralization and after 1, 7 and

28 days of immersion. The hardness and elastic modulus of the enamel surfaces were calculated using the software provided with the nanoindentation apparatus.

The number of samples was 100. One-way analysis of variance and Tukey test were used for statistical analysis ($p < 0.05$).

4) SEM observations of enamel surfaces

Enamel samples before and after the immersion test were observed using SEM.

5) Antibacterial test

The minimum bactericidal concentration (MBC) was measured by the microdilution method in 96-well plate. The same materials used as in the remineralization test, each was diluted to 10%. Ten types of bacterias (*S.mutans, S.sanguinis, S.salivarius, S.gordonii, S.aureus, A.actinomycetemcomitans, P.gingivalis, F.nucleatum, P.intermedia, C.albicans*) were selected. These bacterial solutions were prepared with the BHI liquid culture medium to the turbidity of $OD_{600nm}=1.0\times10^{-3}$, and 100 μL of each bacterial solution was incubated into a plate. 3 μL of each well was incubated the BHI agar medium, and MBC was evaluated based on the presence or absence of bacterial growth.

【Results and Discussion】

As a result of the characterization, it was confirmed that the produced particles(nB, C) were circular particles having an amorphous structure, and very large specific surface area by nanoparticulation, moderate dispersion stability because of surface charge. Furthermore, after immersion of nB in an acetic acid, the pH rose rapidly and reached about 10 at a concentration of 5 mg/mL or higher, indicating excellent acid buffering capacity.

All the enamel-embedded samples showed a significant decrease in surface mechanical properties due to decalcification by 35% phosphoric acid etching. After that, remineralization test showed a faster recovery of mechanical properties of the enamel surface to which the bioactive glass nanoparticles-containing group (nB, nBC, nBZ, nBCZ) was applied. These showed significantly better recovery than Con and F

In observation of the surface texture by SEM, the honeycomb-like and rough, porous surface structure of decalcified enamel was covered with remineralization-like structure after the remineralization test in all groups.

MBC in the zinc oxide-containing group (nBZ, nBCZ) were identified for all bacterias including the cariogenic bacteria (*S. mutans*). It was confirmed that it has a broad-range antibacterial spectrum.

【Conclusion】

The application of bioactive glass and zinc oxide composite nanoparticles probably promotes enamel remineralization and provides antibacterial activity against oral bacteria.